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BIOTECHNOLOGICAL APPLICATIONS OF PURPLE NON SULPHUR PHOTOTROPHIC BACTERIA: A MINIREVIEW

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Bacteria play in vital role in the production of variety of products, including certain plastics and enzymes used in detergents, textiles and pharmaceutical industries. Production of chemicals using bacteria and other microorganisms is not only economical sustainable but also ecofriendly. Modern biotechnology entails the use of cell fusion, bioinformatics, genetic engineering, structure based molecular design and hybridoma technology. The presence of photosynthetic bacteria along with the heterotrophic bacteria have been reported in various aquatic environments like Indian tropical waters (Vasavi *et al*., 2007), salt marshes (Bergstein *et al*., 1993), industrial effluents (Ramasamy *et al*., 1990; Merugu *et al*., 2008), sea water (Kobayashi,1982), sewage (Kobayashi *et al*., 1995), waste water (Sunita and Mitra, 1993 and Vasavi *et al*., 2007), hot water springs (Demchick *et al*., 1990), earthworm casts (Vasavi *et al*., 2007), paddy fields (Sasikala *et al*., 2004), ocean waters and aquaculture (Kappler *et al*.,2005), brackish lagoon (Anthony *et al*., 2006), and black sea (Overmann and Manske, 2006). Blankenship *et al*. (1995) studied taxonomy of anoxygenic photosynthetic bacteria.

These bacteria preferably grow by a photoheterotrophic metabolism with organic substances as electron donors during their photosynthetic activity. Most species are also capable of growing photoautotrophically with molecular hydrogen as donor. They have high protein content with good amount of essential amino acids, vitamins, biological co-factors and fewer amounts of nucleic acids (Sasikala and Ramana, 1995, Merugu et al.,2008). Many workers have recommended purple non sulphur bacteria as a source of SCP for pisciculture and poultry industry (Salma *et al*., 2007), vitamin B_{12} (Sasikala and Ramana,1995), Ubiquinone Q_{10} used in clinical medicine (Sasaki *et al*., 2002) and therapeutically used compounds (Nagumo *et al.,* 1991). Mitsui (1985), while discussing multiple utilisation of tropical and subtropical marine photosynthetic organisms, suggested that some photosynthetic bacterial strains may prove to be an economical source of carbohydrate material for bacterial mediated methane production. Carotenoids produced by *Rb.sphaeroides* are used as natural dyes and food dyes (Qian *et al.,* 1991). The profile of essential amino acids of anoxygenic phototrophic bacteria is comparable to those of soyabean and egg proteins (Ponsanio *et al*., 2002) and higher than those of other single cell protein (Azad *et al*., 2001). Mass production of *Rhodopseudomonas palustris* as diet for aquaculture was studied by Kim and Lee (2000). Many of phototrophic bacteria are reported to leach out ammonia during their growth as amino acids. Ammonia leaching was observed in resting cells of *Rhodobacter sphaeroides* O.U.001 in the absence of MSX under various gas phases (Sasikala and Ramana, 1990). Hiroo (2004) used a mixed culture of photosynthetic bacteria for ammonia leaching.

Photosynthetic bacteria are the most potent microorganisms for the production of porphyrins. Ishi *et al*. (1990) reported increased production of porphyrins using immobilized photosynthetic bacteria under illumination *Rb.sphaeroides* CR386 could produce porphyrins under aerobic conditions (Utsunomiya *et al*., 2003). Large amounts of amino levulinic acid excretion by the photosynthetic bacteria has been reported by Sasaki *et al*. (1991). *Rhodovulvum spp* S88 strain could produce extra cellular polymeric substance (EPS) in the surface of its cell (Watanabe *et al*., 1998). Production of EPS by *Rps.acidophila* was also reported (Sheng *et al*., 2006). Production of Indole acetic acid by phototrophic bacteria was reported by many workers including Sasikala and Ramana (1995), Rajasekhar *et al*. (1999), and Merugu *et al*. (2007, 2011). Photobiotransformation of indole acetic acid by some of the purple non sulphur bacteria indicates their role in the production of phytohormones (Sasikala and Ramana, 1995). A new phytohormone rhodestrin was isolated as a metabolite of anthranilate photobiotransformation by *Rhodobacter sphaeroides* OU5 (Sunayana *et al*., 2005).

Table 1: Biotechnological Applications of Purple non sulphur bacteria

S.No	Product	Applications	Reference
1	Single cell protein	Protein source	Sasaki *et al*.(1998)
			Ponsano *et al*.(2003),
2	S-adenosyl	Precursor useful in	Yamada *et al*.(1986
	cystine	therapeutic marketing	
3	Metal ion uptake	Metal recovery	Sanaa *et al*.(2006)
4	Vit B12	Vitamin	Goldrick (2003)
5	Hydrogen	Fuel	Sasikala *et al*.(1992)
			Fedorov et al.(1998)
			Katsuda *et al*.(2000)
			Mahakhan *et al*.(2005)
			Zabut *et al*.,(2005)
6	NH3	Fertilizer	Sasikala and Ramana(1990)
7	Carotenoid	Natural dye	Nopartnarporn *et al*.,(1986)
8	5-Amino levulinic cid	Herbicide	Sasaki *et al*.(1997)
			Kamiyama *et al*(.2000)
9	Biodegration of organic and inorganic compounds	Waste water treatment	Blasco and Castillo,(1993), Merugu et al.,2008, Vasavi etal.,2008
			James *et al*.,(2000),
			Ramana *et al*(2000)
			Nagadomi *et al*.(2000)
			Ramana and Sasikala (2002)
			Takeno *et al*.(2005)
10	Hopanoids	Therapeutic	Nagumo *et al*.,(1991)
11	Poly β hydroxy Alkanaotes	Biodegradable plastic	Brandl *et al* (1991),
			Serdyuk *et al*.,(1993)
			Suzuki *et al*.,(1995)
			Hashimoto *et al* (1993),
			Kranz *et al*.(1997)
			Mahuya *et al*.(2005)
			Lorrungruang, (2006)
12	Enzymes	Food preparations	Scavetta *et al*.(2000)
		and Biotechnology	Srinivas *et al*.,(2007)
13	Cytokinins	Plant growth	Serdyuk *et.al*.(1993)
		hormones	Scavetta *et al*.(2000)
14	Hydrogen	Fuel	Sasikala and Ramana(1991), Merugu et al.(2010,2011a,2012)

Many members of the Rhodospirillaceae show a high resistance towards toxic heavy metal oxides and oxyanions (Moore *et al*., 1992). *Rhodobacter sphaeroides* and *Rhodovulvum sp*. are capable of cadmium removal in a batch culture system (Watanabe, 2003). Removal of phosphorus from oyster farm mud sediment using *Rhodobacter sphaeroides* Il 106 was reported by Takeno *et al*. (1999). *Rsp. rubrum* is reported to reduce and detoxify elemental selenium (Kessi *et al*., 1999). Biosorption characteristics of cadmium and lead ions of *Rhodobacter sphaeroides* and *Alcaligenes eutrophus* H16 were discussed by Seki (1998). Phototrophic bacteria were also reported to show considerable resistance towards heavy metals (Merugu et al-., 2008). Trace elements usually act as cofactors for essential reactions in the cell. *Rhodobacter sphaeroides* is reported to produce maximum amount of hydrogen in presence of molybdenum (Kars *et al*., 2005). Zinc and cadmium are reported to be toxic to *Rb.sphaeroides* (Balasobre *et al*., 2006) while nickel and cobalt were reported to decrease the cellular content of the light harvesting complexes. On the other hand *Rhodobacter sphaeroides* was highly tolerant to metal exposure especially towards cobalt, iron and molybdenum (Giottia *et al*., 2006).

Anoxygenic phototrophic bacteria are the major groups of microorganisms existing in paddy soils and contribute significantly to soil fertility (Hable and Alexander, 1980). Chalam *et al*. (1992) have reported the adverse effect of pesticide on diazotrophic growth and nitrogenase activity of some anoxygenic phototrophic bacteria isolated from paddy soils. On the other hand carbendazim was shown to be photo assimilated by *Rps.palustris* strain as sole carbon and nitrogen source (Rajkumar and Lalitha Kumari, 1992). Tolerance of phototrophic bacterium towards certain pesticides was reported by Merugu et al. (2008). *Rhodoferax* species was reported to degrade phenoxy herbicides (Ehrig *et al*., 1997). Berne *et al*. (2005) have reported tributyl phosphate (TBP) degradation by *Rps.palustris* and other photosynthetic bacteria . Poly β-hydroxy butyrate (PHB) is an unique intracellular polymeric material which gets accumulated under unbalanced growth conditions in a wide variety of bacteria. Combination of various carbon and nitrogen substrates were tried for PHB accumulation and hydrogen evolution in *Rb.sphaeroides* strain RV. An increase in pH caused an increase in PHB accumulation on lactate under nitrogen deprived conditions (Katipov *et al*., 1998). PHB production under different cultural conditions such as nitrogen, phosphate and sulphate limitations was reported by Merugu et al.,2010a,2010b). Polyhydroxy alkanoate (PHA) production by *Rb.sphaeroides* and *Rb.sphaeroides* IL 106 from acetic acid was reported by Noparatnaraporn (2001). Lorrungruang (2006) feels that a photosynthetic bacterium that can produce PHA and grows well at 37-40°C in an aerobic dark condition is ideal for this purpose. Brandl *et al*. (1991) reported that *Rb.sphaeroides* produced PHB as the major component (97%) and a small amount of PHV (3%) under anaerobic light conditions.

Biofertilisers, more commonly known as microbial inoculants, are artificially multiplied cultures of certain soil organisms that can improve soil fertility and crop productivity. Madian *et al*. (1981) showed that in the absence of combined nitrogen but in the presence of diazotrophs like *Azotobacter vinelandii* and *Rhodopseudomonas capsulatus* can benefit rice plants for nitrogen nutrition upto ear stage. Elbadry and Elbanna, (1999) have demonstrated beneficial effect of *Rhodobacter capsulatus* on four rice varieties in hydroponic cultures. Application of freeze dried purple non sulphur bacterium *Rhodobacter sphaeroides* resulted in improvement in the fruit quality of tomato fruit (Kondo, 2006). Photosynthetic bacteria are reported to produce hydrolytic enzymes which can be exploited industrially (Merugu et al,2011). Anoxygenic phototrophic bacterial amylases produce maltooligossacharides which are potentially useful in food pharmaceutical and chemical industries. Amylase produced by anoxygenic phototrophic bacteria could hydrolyze oyster glycogen, amylose and pullulan apart from soluble starch (Burankarl *et al*., 1998). Srinivas *et al*. (2003) reported the production of amylase by *Rhodocyclus gelatinosus*. Isaki and Kanmio (1992) produced a novel enzyme that could modify skim milk beverages.

Of various methods of hydrogen production such as electrolysis of water, photoelectrolysis, photocatalysis and biophotolysis, phototrophic production of hydrogen proved to be far superior. Light dependant hydrogen production by anoxygenic photosynthetic bacteria (Vatsala, 1990; Sasikala *et al.,* 1993; Burgess *et al.,* 1994; Edward and Andrew, 2002,Merugu et al.,2010a,2011a,2011b) and cell free artificial reconstituted systems (Kumazawa and Shimamura, 1993) was reviewed by many workers.

The production of hydrogen would not involve the evolution of pollutants (Melis, 2002) as in the case of fossil fuel refineries. The hydrogen produced would be clean burning yielding water. Utilization of waste water for photobiological hydrogen generation by photosynthetic purple non sulphur bacteria (Sunita and Mitra, 1993; Thangaraj and Kulandaivelu, 1994; Melis, 2002; Lee *et al*., 2002; Hoekema *et al*., 2002; Vasavi *et al*.,2008; Merugu *et al*., 2008) is desirable since it not only makes the process of photobiological hydrogen generation operationally feasible but also achieves partial purification of water by reducing organic materials (Ramchander *et al*., 2007 and Vasavi *et al*., 2008). Melis (2005) suggested a plan for integrated biological production of hydrogen. Generation of hydrogen from waste waters from the food industry was studied by Marek (2007). Survey of various carbon sources on hydrogen production by *Rsp.rubrum* was studied by Najafpour (2006). *Rhodopseudomonas palustris* CGA 09 was immobilized in thin nonporous latex, produced good amount of hydrogen in an argon atmosphere (Gosse *et al*., 2007). When incubated in the presence of CO gas, *Rubrivivax gelatinosus* CS induced a CO oxidation hydrogen production pathways which proceeds in both light and darkness (Maness *et al*., 2005). Fermentative production of hydrogen from synthetic gas was investigated by Najafpour (2006).

Changes in physico-chemical parameters and photosynthetic microorganisms in a deep waste water self depuration lagoon have been reported by Soler *et al*. (1991). Recent molecular studies techniques such as Fluorescence *in situ* hybridization (FISH) analysis showed that bacteria of the beta-2 sub class of proteobacteria and acinetobacteria have extremely good phosphorus removal capacity (Bond *et al*., 1995). Purple non sulphur bacterium *Rhodobacter capsulatus* immobilized on cellulose beads removed organic carbon, ammonium ions and phosphate ions from a diluted growth medium over a period of 19-22 days (Sawayama *et al*., 1998). *Rhodobacter sphaeroides, Rhodobacter sphaeroides* NR-3 and *Rhodopseudomonas palustris* immobilized in porous ceramic removed phosphates, nitrates and H_2S from synthetic waste water (Nagadomi *et al*., 2000). Julie *et al*. (2002) investigated phosphorus removal by *Rhodocyclus* spp. Several workers reported the use of anoxygenic phototrophic bacteria in the degradation of organic wastes mostly agricultural and food industries which include molasses wastes (Sasikala *et al.,* 1992), citric acid fermentation wastes (Yu *et al.,* 1991), refinery, lactic acid wastes (Sasikala *et al.,* 1995), high strength organic waste water (Ogbonna, 2000) and oil containing sewage water (Takeno *et al.,* 2005). Hassan *et al*. (1997) have reported the efficient treatment of palm oil by *Rhodobacter sphaeroides*. Treatment of oil containing sewage waste water using immobilized photosynthetic bacteria was reported by Takeno *et al*. (2005). *Rps. palustris* could degrade tributyl phosphate which is widely used in nuclear fuel processing and other waste generating chemical industries (Berne *et al*., 2005). Merugu et al. (2008) have shown phosphate solubolisation by this phototrophic bacteria studued by them. Ibrahim *et al*. (2006) have studied different factors influencing depollution of sago effluent by *Rps.palustris* strain B1. Phototrophic bacteria degrade aromatic compounds such as benzoates, benzoate derivatives (Wright and Madigan, 1991), amines (Cabello *et al*., 2004), thiols (Visscher and Taylor, 1993) and phenols (Blasco and Castello, 1993). Mixed culture of *Rb.sphaeroides*, *Chlorella sorokiniana* and *Spirulina platensis* proved to be better for treating organic waste water (Ogbonna, 2000). Due to the widespread use, chromium is considered to be a serious environmental pollutant. Cr (VI) is highly toxic whereas Cr (III) is less toxic. Chromate reduction was detected in *Rhodobacter sphaeroides* (Nepple *et al*., 2000) and *Rhodobacter capsulatus* KU002 (Merugu et al., 2011)

Genetic transfer systems are powerful tools in genetic analysis and essential for the construction of recombinant bacteria. Ribulose 1,5 phosphate carboxylase gene from *Rsp. rubrum* was expressed in *E.coli* to study the effect of oxygen on its regulation (Cook and Tabita, 1988). Gene encoding transcription termination *rho* from *Rhodobacter sphaeroides* 2.4.1 was cloned into *E.coli* and the deduced protein was sequenced (Gomelsky and Kaplan, 1996). DNA repair genes *rec* A from *Rhodopseudomonas viridis* was transferred to *rec-* phenotype of *Rhizobium melitoli* (Chen and Michel, 1998). *E. coli* was transformed using a *hem* c gene of *Rb. capsulatus* coding for a porphobilinogen deaminase (Beil *et al*., 2002). Biological rhythms of gene expression in a purple bacterium, *Rhodobacter sphaeroides* by using a luciferase reporter gene system was reported by Min *et al*. (2005).

Purple non sulphur bacteria are the most diverse and versatile of all photosynthetic bacteria with vast potentials for applications. Hence, in view of their abilities to produce biohydrogen, bioplastic, bioherbicides, phytohormones and industrially important enzymes they can be exploited for industrial production of these products.

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